Iranian Journal of Veterinary Science and Technology
Vol. 3, No. 2, 2011, 49-56

IJVST

A case report of atypical borreliosis in a dog

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Received: June 7, 2009 Accepted: November 22, 2009

Abstract

Borreliosis is a bacterial zoonosis transmitted by arthropods. The infection is more common in dogs than other domestic animals. A one-month-old puppy with fever, jaundice and digestion disorder was referred to Small Animal Teaching Hospital, University of Tehran. Laboratory findings included severe regenerative anemia, neutrophilia, thrombocytopenia, strong spirochetemia and significant changes in serum proteins. Attempt to isolate the bacteria in laboratory animals was unsuccessful. The infection was cured using antibiotics and the animal was completely recovered.

Keywords: Borreliosis, zoonosis, dog, arthropod, tick, Iran

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Introduction

Borreliosis is an endemic zoonosis mostly transmitted by ticks and possibly other arthropods such as fleas and bloodsucker insects (Fritz and Kjemtrup, 2003). The causative agent is classified in Order Spirochaetales, Family Spirochaetaceae and Genus *Borrelia* (Paster and Dewhirst, 2000). *Borrelia* spp. are Gram-negative, spirilliform and motile bacteria that are host dependent and are not found in nature (Steere, 2001). At least four species—*Borrelia persica*, *B. microti*, *B. baltazardi* and *B. latyschewii*—have been identified in Iran, mainly transmitted by Argasidae soft ticks (Nekoei and Asmar, 1996, 1993; Barbour and Hayes, 1986). These ticks infect mammals such as dogs, cats and humans while bloodsucking (Bushmich, 1994). Dogs are the most infected animals amongst the pets (Guerra *et al*., 2001). The potential risk of simultaneous transmission of other genera such as *Leptospira*, *Treponema*, *Ehrlichia*, *Babesia* and *Giardia* with *Borrelia* increases the importance of the disease (Skarda, 2005; Mullegger, 2004). Estimation of infected ticks in city suburbs such as parks, playgrounds and rural counties is necessary for the evaluation of borreliosis risk in societies (Guerra *et al*., 2001). Light or darkfield microscopy is used in dogs for diagnosis of *Borrelia* infections in addition to silver staining and serological assays such as enzyme-linked immunosorbent assay (ELISA), Western blot, and immunofluorescence antibody techniques (FAT) (Appel *et al*., 1993; Golightly *et al*., 1990). Molecular techniques such as polymerase chain reaction (PCR), genome sequencing and fingerprinting are more accurate but need special methods and facilities (Wang *et al*., 1999). Bacterial isolation and culture represent the most reliable methods for the detection of *Borrelia* spp. but are not easily performed (Nielssen *et al*., 2002). Treatment scheme is based on the use of tetracyclines or penicillins (Przytula *et al*., 2006). Oral doxycycline and amoxicillin are the most common antibiotics used in usual cases while injection ceftriaxone (third-generation cephalosporin) is used in chronic and resistant infections or in hypersensitive cases (Duffy, 1990, 1987). Furthermore, *Borrelia* spp. is sensitive to erythromycin (alternative option) and chloramphenicol. Antibiotics should be prescribed for at least 14 days but are recommended to continue for 30 days (Garbe, 1991); however, they do not eradicate the bacteria (Straubinger *et al*., 1997). Use of corticosteroids such as prednisolone for two weeks helps to eliminate disease symptoms and reduces the pain, promising more relief for the patients (Straubinger *et al*., 2000). Prophylaxis of borreliosis is based on the vaccination and tick control programs including avoid of contaminated area, use of appropriate cover, body check and tick removal, use of acaricides and bush trimming (Patey, 2007). Antibiotic prophylaxis is still not known to be quite effective and does not guarantee an ideal prevention (Mullegger, 2004).

Case Presentation

A one-month-old female mixed puppy with diarrhea, malaise, anorexia and pustules was referred to Small Animal Teaching Hospital, Faculty of Veterinary Medicine, University of Tehran. The puppy was suffering from poor health condition and had been attacked by many ectoparasites such as lice and soft ticks. No history of living condition, vaccination or treatment was available except that the animal was kept in a rural area with three siblings. Serial blood samples were collected and sent to clinical pathology laboratory. Later, other puppies were clinically examined and blood samples were taken. All cases were followed up for a few weeks.

Results

Fever, icteric mucosa and pain on palpation were found during the clinical examinations. No neuropathy, ophthalmopathy, arthritis or lameness was diagnosed. Laboratory findings included severe regenerative anemia

(anisocytosis, hypochromia and reticulocytosis), neutrophilia (toxic neutrophils), thrombocytopenia and strong spirochetemia in direct microscopy of Giemsa-stained blood smears (Figures 1 and 2). The hemogram included erythrocyte, 9.1×10^5/µl; hemoglobin, 3.8 g/dl; hematocrit, 12%; MCV, 133.3 fl; MCH, 42.2 pg; and MCHC, 31%. The leukogram included leukocytes, 9.75×10^3/µl; neutrophils, 87%; band cells, 1%; lymphocytes, 12%; monocytes, 0%; and eosinophils, 0%. Significant protein changes were shown in serum electrophoresis, including decreases in albumin (1.92 g/dl), alpha-1 (0.17 g/dl), beta (1 g/dl) and gamma (0.49 g/dl) fractions. Alpha-2 fraction (0.82 g/dl) was reported normal. Total protein value was subnormal (4.4 g/dl), while A/G ratio (0.78) was unchanged. The attempt to isolate the bacteria in sensitive laboratory animals was unsuccessful. Hematology of other puppies revealed a better situation as 2 puppies had normal blood values (including a mild regenerative anemia) and lacked spirochetemia. Spirochetes were seen in blood samples of the fourth puppy but the number of bacteria was significantly low. Since no special report had been published on antibiotic resistance in *Borrelia* spp., the treatment protocol was planned based on the recommended antibiotics. Doxycycline (10 mg/kg, PO, BID for 30 days) and prednisolone (30 mg/kg, PO, SID for 7 days) were prescribed. Bacteremia was eradicated from the peripheral blood shortly after the use of antibiotic and the animal's general condition seemed better. In follow up, no bacteria were seen in direct microscopy and the blood values turned to normal. No clinical manifestation or sign of fever, anorexia, diarrhea or pain was seen and the animal regained her lost weight. Drug regiment and results for other puppies were mostly the same.

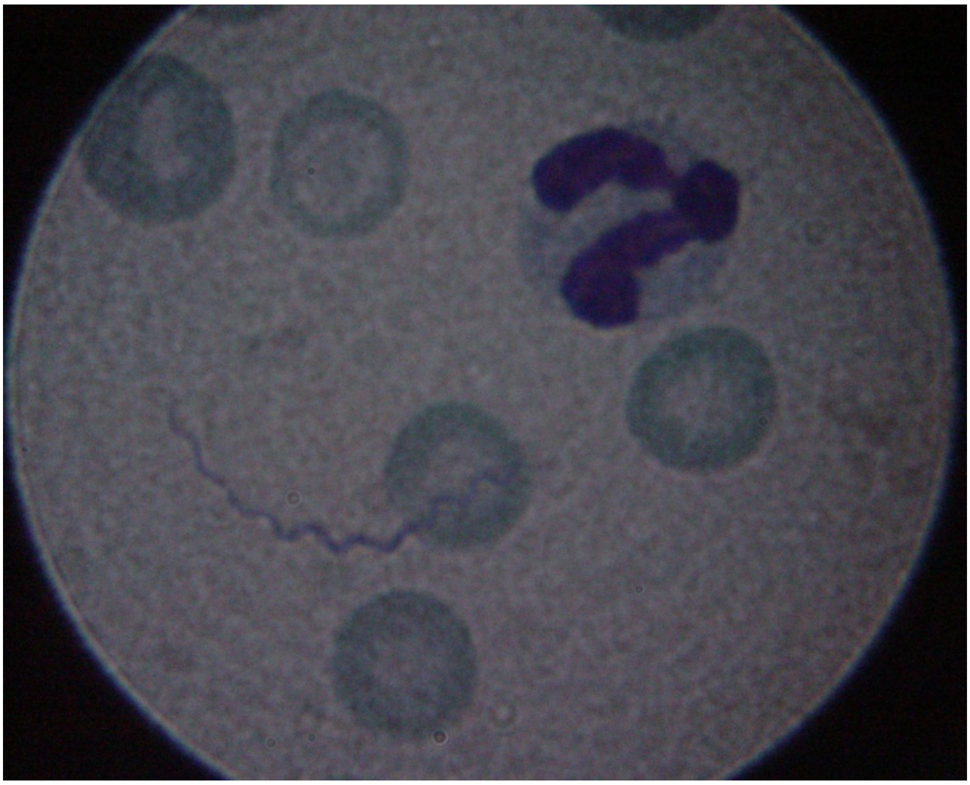


Figure 1. *Borrelia* spp. in blood smear (100×). Upright: a toxic neutrophil with basophilic cytoplasm.

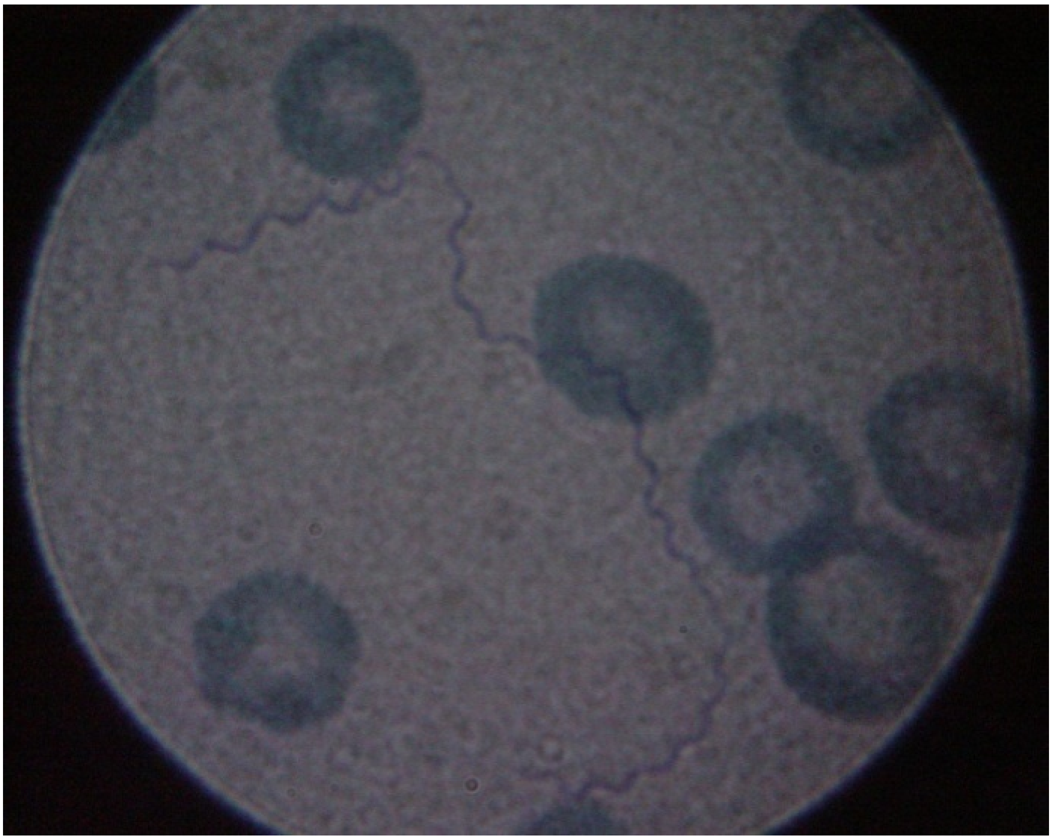


Figure 2. ***Borrelia*** **spp. in blood smear (100×). RBC morphology shows anisocytosis and hypochromia.**

Discussion

Regarding to literature reviews, borreliosis is mostly prevalent in dogs rather than other animals (Eng *et al*., 1988). Despite its heavy infection, the present case lacked typical symptoms and only showed general symptoms including fever, anorexia and malaise. Although puppies usually present symptoms sooner than adults do, some infected animals may not present clinical symptoms (Appel *et al*., 1993). Experiments have shown that *Borrelia* is potentially able to stimulate a broad spectrum of host cells such as endothelials, monocyte/macrophages, lymphocytes and neutrophils (Hodzic *et al*., 2008); therefore, the neutrophilia of the case is sensible. Other abnormal blood values, including lymphopenia and eosinopenia, may occur in response to endogenous glucocorticosteroids (Whitney *et al*., 2007; Jackson *et al*., 2007). Chioa *et al*. (2000) studied the preventive effect of *Borrelia* OspA surface antigen on lymph cell proliferation resulting in lymphopenia.

Anemia and thrombocytopenia are other laboratory findings in borreliosis (Shaw *et al*., 2005); both seen in this case. Unlike recent report by Whitney *et al*. (2007) that indicates degenerative anemia in dogs with borreliosis, the patient had macrocytic hypochromic regenerative anemia. This difference seems to link to the sufficient time needed for the activation of bone marrow hematopoiesis and release of reticulocytes into the bloodstream following the anemia onset; as the animal's blood values shifted to normal during the disease. Despite the laboratory findings that suggest an increase in blood proteins and

gammaglobulins (Scorpio *et al*., 2008), the animal suffered from a general decrease in serum proteins. The albumin deficiency matches to a study by Whitney *et al*. (2007) that reports 2.5 mg/dl albumin in dogs with borreliosis. Direct microscopy of Giemsa-stained blood smears and observation of spirochetes seem to be the only available diagnostic tools in the case of atypical borreliosis because *Borrelia* spp. are not usually suspected in infections lacking the key signs. Furthermore, nonspecific test results (e.g. ESR, CBC and hepatic enzymes) are not practically reliable because they are subjected to change (Steere *et al*., 1983). However, the viability of this method in asymptomatic dogs is questioned because spirochetemia is found in healthy dogs as well (Greene, 1991). Since most of the commercial ELISA kits and fluorescent antibodies are designed against *B. burgdorferi*, evaluation of serum antibodies in areas contaminated by other species is uncertain, especially in absence of a proper diagnostic assay. On the other hand, no accurate data on the prevalence of *Borrelia* spp. are available in many areas due to the lack of a comprehensive survey plan.

Therefore, establishment of a *Borrelia* survey and monitoring program is necessary to prevent and control the infection and to assist the patients. Unfortunately, blood findings have not already been concerned in borreliosis and most of the papers have focused on borreliosis clinical symptoms and manifestations, evaluation of serum analysis or molecular assays for the disease (Littman, 2003). Therefore, papers that focus on hematology, urinalysis and other similar subjects will generously support the development of a practical diagnostic protocol, especially in case of atypical borreliosis.

Acknowledgment

We wish to thank staffs within the Small Animal Teaching Hospital for their kind help.

References

Appel, M. J., Allan, S., Jacobson, R. H., Lauderdale, T. L., Chang, Y. F., Shin, S. J., Thomford, J. W., Todhunter, R. J., and Summers, B. A. (1993) Experimental Lyme disease in dogs produces arthritis and persistent infection. *The Journal of Infectious Diseases* **167**, 651–64.

Barbour, A. G., and Hayes, S. F. (1986) Biology of *Borrelia* species. *Microbiological Reviews* **50**, 381–400.

Bushmich, S. L. (1994) Lyme Borreliosis in domestic animals. *Joural of Spirochet Tick Disease* **1**, 24−28.

Chiao, J. W., Villalon, P., Schwartz, I., and Wormser, G. P. (2000) Modulation of lymphocyte proliferative responses by a canine Lyme disease vaccine of recombinant outer surface protein A (OspA). *FEMS Immunology and Medical Microbiology* **28**, 193−196.

Post, J. E., Shaw, E. E., and Wright, S. (1986) Suspected borreliosis in cattle. *Annals of the New York Academy of Sciences* **539**, 488.

Duffy, J. (1987) Lyme disease. *Infectious Disease Clinics of North America* **1**, 511–27.

Duffy, J. (1990) Lyme disease. *Annals of Allergy* **65**, 1–13.

Eng, T. R., Wilson, M. I., Spielman, A., and Lastavica, C. C. (1988) Greater risk of *Borrelia burgdorferi* infection in dogs than in people. *Journal of Infectious Disease* **158**, 1410−11.

Fritz, C. L., and Kjemtrup, A. M. (2003) Lyme borreliosis. *Journal of the American Veterinary Medical Association* **223**, 1261−1270.

Garbe, C. (1991) *Borrelia* infections of the skin--progress of knowledge since the discovery of Lyme disease. *Hautarzt* **42**, 356–65.

Golightly, M. J., Thomas, A., and Viciana, A. L. (1990) The laboratory diagnosis of

Lyme borreliosis. *Laboratory Medicine* **21**, 299−304.

Greene, R. T. (1991) Canine Lyme borreliosis. Veterinary Clinics of North America: *Small Animal Practice* **21**, 51–64.

Guerra, M. A., Walker, E. D., and Kitron, U. (2001) Canine surveillance system for Lyme borreliosis in Wisconsin and northern Illinois: geographic distribution and risk factor analysis. *The American Journal of Tropical Medicine and Hygiene* **65**, 546−52.

Hodzic, E., Feng, S., Holden, K., Freet, K. J., and Barthold, S. W. (2008) Persistence of *Borrelia burgdorferi* following antibiotic treatment in mice. *Antimicrobial Agents and Chemotherapy* **52**, 1728–1736.

Jackson, S., Gilchrist, H., and Nesbitt, L. T. (2007) Update on the dermatologic use of systemic glucocorticosteroids. *Dermatology and Therapy* **20**, 187–205.

Littman, M. P. (2003) Canine borreliosis. *Veterinary Clinics of North America Small Animal Practice* **33**, 827–62.

Mullegger, R. R. (2004) Dermatological manifestations of Lyme borreliosis. *European Journal of Dermatology* **14**, 296–309.

Nekoei, H., and Asmar, M. (1993, 3 December) The new studies on borreliosis in Pasteur Institute of Iran. The 2nd *National* Congress of Zoonoses in Iran, *Tabriz, Iran.*

Nekoei, H., and Asmar, M. (1996) Distribution of borreliosis in Iran. *The 3rd National Congress of Zoonoses in Iran, Mashhad, Iran. pp.23−25*

Nielssen, A., Carr, A., and Heseltine, J. (2002) Update on canine Lyme disease. *Veterinary Medicine* **1**, 604–609.

Paster, B. J., and Dewhirst, F. E. (2000) Phylogenetic foundation of spirochetes. *Journal of Molecular Microbiology and Biotechnology* **2**, 341–344.

Przytula, L., Gindzienska-Sieskiewicz, E., and Sierakowski, S. (2006) Diagnosis and treatment of Lyme arthritis. *Przegl Epidemiol*ogy **60** Suppl (1), 125–30.

Scorpio, D. G., Wachtman, L. M., Tunin, R. S., Barat, N. C., Garyu, J. W., and Dumler, J. S. (2008) Retrospective clinical and molecular analysis of conditioned laboratory dogs (Canis familiaris) with serologic reactions to Ehrlichia canis, Borrelia burgdorferi, and Rickettsia rickettsii. *Journal of the American Association for Laboratory Animal Science* **47**, 23−8.

Shaw, S. E., Binns, S. H., Birtles, R. J., Day, M. J., Smithson, R., and Kenny, M. J. (2005) Molecular evidence of tick-transmitted infections in dogs and cats in the United Kingdom. *Veterinary Record* **157**, 645−8.

Skarda, J. (2005) Lyme borreliosis, the present diagnostic criteria. *Folia Veterinaria* **49**, S33−S34.

Steere, A. C. (2001) Lyme disease. *The New England Journal of Medicine* **345**, 115−125.

Straubinger, R. K. (2000) PCR-based quantification of *Borrelia burgdorferi* organisms in canine tissues over a 500-day postinfection period. *Journal of Clinical Microbiology* **38**, 2191–2199.

Straubinger, R. K., Straubinger, A. F., Summers, B. A., and Jacobson, R. H. (2000) Status of *Borrelia burgdorferi* infection after antibiotic treatment and the effects of corticosteroids: an experimental study. *Journal of Infectious Diseases* **181**, 1069–1081.

Straubinger, R. K., Summers, B. A., Chang, Y., and Appel, M. J. G. (1997) Persistence of *Borrelia burgdorferi* in experimentally infected dogs after antibiotic treatment. *Journal of Clinical Microbiology* **35**, 111–116.

Wang, G., van Dam, A. P., Schwartz, I., and Dankert, J. (1999) Molecular typing of *Borrelia burgdorferi* sensu lato: taxonomic, epidemiological, and

clinical implications. *Clinical Microbiology Reviews* **12**, 633–653.

Whitney, M. S., Schwan, T. G., Sultemeier, K. B., McDonald, P. S., and Brillhart, M. N. (2007) Spirochetemia caused by *Borrelia turicatae* infection in 3 dogs in Texas. *Veterinary Clinical Pathology* **36**, 212−6.

Iranian Journal of Veterinary Science and Technology
Vol. 3, No. 2, 2011, 49-56

IJVST

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دریافت مقاله : ۱۳۸۸/۳/۱۷ پذیرش نهایی : ۱۳۸۸/۹/۱

چکیده

بورلیوز بیماری مشترک اندومیکی است که بوسیله کنه منتقل می‌شود. در میان حیوانات خانگی سگ‌ها بیشتر به بورلیوز دچار می‌شوند. یک توله سگ ماده مخلوط یک ماهه از مجموع چهار توله با علائم تب، زردی و مشکلات گوارشی به بیمارستان آموزشی حیوانات کوچک دانشکده دامپزشکی دانشگاه تهران ارجاع شد. یافته‌های آزمایشگاهی شامل کم خونی شدید جبران پذیر، نوتروفیلی، ترومبوسایتوپنی، اسپیروکتمی نسبتاً شدید و تغییرات شدید در پروتئین های سرم بوده است. تلاش برای جداسازی باکتری در حیوان آزمایشگاهی ناموفق بود. عفونت با استفاده از آنتی بیوتیک درمان شده و حیوان بهبود یافت.

واژگان کلیدی: بورلیوز، بیماری مشترک، سگ، بندپایان، کنه، ایران

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